The Trypanosome causing Disease in Man in Nyasaland: The Liwonde Strain. Part I.—*Morphology.* Part II.—*Susceptibility of Animals.*

By Surgeon-General Sir David Bruce, C.B., F.R.S., A.M.S.; Major A. E. Hamerton, D.S.O., and Captain D. P. Watson, R.A.M.C.; and Lady Bruce, R.R.C. (Scientific Commission of the Royal Society, Nyasaland, 1912–14.)

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Introduction.

This strain was obtained in the "fly-area" of the Upper Shiré Valley, in the Liwonde district, which is situated about 100 miles south of the "Proclaimed Area."* At the time it was procured no cases of trypanosome disease in man had been reported from this district; lately, a case has been discovered at Mpimbi, in the south of the district, about 150 miles south of the "Proclaimed" or Sleeping Sickness Area.

Three dogs infected with the wild *Glossina morsitans* strains of this trypanosome were brought to Kasu, and other animals—monkeys, dogs and rats—were inoculated from them.

For purposes of description, measurement and comparison, only trypanosomes from rats were used.

I. *Morphology of the Liwonde Strain.*

A. *Living, Unstained.*

All three strains agree in being actively moving flagellates, but with little or no translatory movement.

B. *Fixed and Stained.*

The blood films were fixed, stained and measured as previously described in the 'Proceedings.'†

Before proceeding to describe the three strains in detail, it may be stated here that in general appearance, shape, position of nucleus, size of micronucleus, contents of cell, and undulating membrane, all three strains were similar, and in no way differed from the various strains of the trypanosome causing disease in man in Nyasaland which have already been described.

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* "Trypanosomes Found in Wild *Glossina morsitans* and Wild Game in the 'Fly-belt' of the Upper Shiré Valley," 'Roy. Soc. Proc.,' B, vol. 88, p. 38 (1914).

† B, vol. 81, pp. 16 and 17.

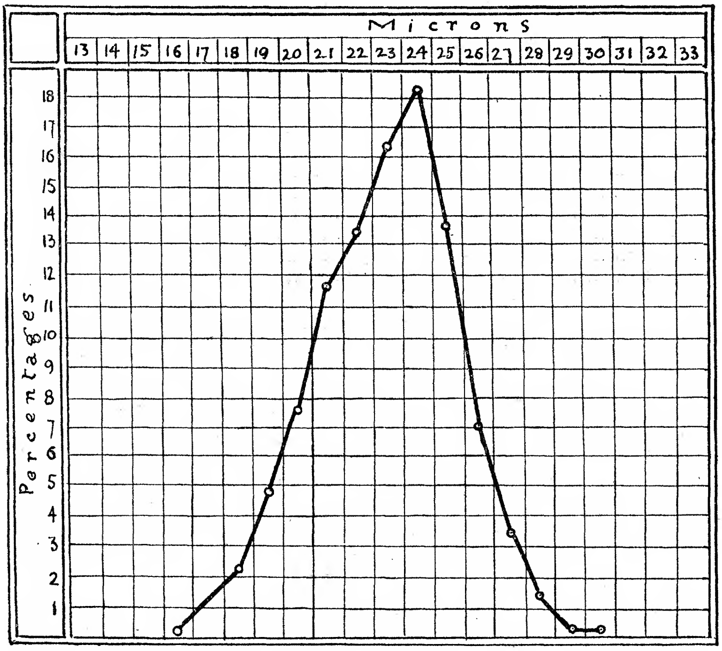
MORPHOLOGY OF THE LIWONDE STRAIN I.

Length.—The following table gives the length of this trypanosome as found in the white rat—500 trypanosomes in all.

Table I.—Measurements of the Length of the Trypanosome of the Liwonde Strain I.

Date.	No. of expt.	Animal.	Method of fixing.	Method of staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
1913.							
Aug. 18...	2378	Rat	Osmic acid	Giemsa	24·8	28·0	19·0
,, 18...	2378	,, ...	,,	,,	24·3	27·0	21·0
,, 18...	2378	,, ...	,,	,,	24·6	27·0	22·0
,, 19...	2378	,, ...	,,	,,	23·4	27·0	20·0
,, 19...	2878	,, ...	,,	,,	23·4	27·0	20·0
,, 19...	2378	,, ...	,,	,,	24·7	27·0	21·0
,, 20...	2378	,, ...	,,	,,	22·3	25·0	19·0
,, 20...	2378	,, ...	,,	,,	21·4	25·0	18·0
,, 20...	2378	,, ...	,,	,,	21·3	24·0	18·0
,, 21...	2378	,, ...	,,	,,	22·1	26·0	16·0
,, 21...	2378	,, ...	,,	,,	22·1	26·0	19·0
,, 21...	2378	,, ...	,,	,,	21·2	25·0	18·0
,, 22...	2378	,, ...	,,	,,	23·3	28·0	20·0
,, 22...	2378	,, ...	,,	,,	23·1	26·0	20·0
,, 22...	2378	,, ...	,,	,,	23·2	28·0	20·0
,, 23...	2378	,, ...	,,	,,	23·3	28·0	21·0
,, 23...	2378	,, ...	,,	,,	24·0	28 0	20·0
,, 23...	2378	,, ...	,,	,,	24·2	30·0	20·0
,, 24...	2378	,, ...	,,	,,	23·0	28·0	20·0
,, 24...	2378	,, ...	,,	,,	23·1	29·0	19·0
,, 24...	2378	,, ...	,,	,,	22·9	25·0	19·0
,, 25...	2378	,, ...	,,	,,	22·5	27·0	18·0
,, 25...	2378	,, ...	,,	,,	22·0	26·0	18·0
,, 25...	2378	,, ...	,,	,,	22·0	25·0	18·0
,, 26...	2378	,, ...	,,	,,	21·8	24·0	18·0
					23 0	30·0	16·0

Chart 1.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Liwonde Strain I, taken on Nine consecutive Days from Rat 2378.



This curve differs from the ordinary Wild *G. morsitans* curve, but is similar to Strains II, IV and V of the Human strain.*

Breadth.—The following table gives the breadth of this trypanosome in the rat—500 trypanosomes in all.

Table II.—Measurements of the Breadth of the Trypanosome of the Liwonde Strain I, measured across the Widest Part, including the Undulating Membrane.

Experiment No.	Animal.	Number measured.	In microns.		
			Average breadth.	Maximum breadth.	Minimum breadth.
2378	Rat	500	3·0	4·50	1·25

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* 'Roy. Soc. Proc.,' B, vol. 86, pp. 288, 295, and 298 (1913).

Table III.—Percentage of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of the Liwonde Strain I.

Date.	Experiment No.	Animal.	Percentage among short and stumpy forms.
1913.			
Aug. 18.........	2378	Rat	2
„ 19.........	2378	„	23
„ 20.........	2378	„	17
„ 21.........	2378	„	4
„ 22.........	2378	„	12
„ 23.........	2378	„	16
„ 24.........	2378	„	28
„ 25.........	2378	„	15
„ 26.........	2378	„	19
„ 27.........	2378	„	22
		Average.........	15·8

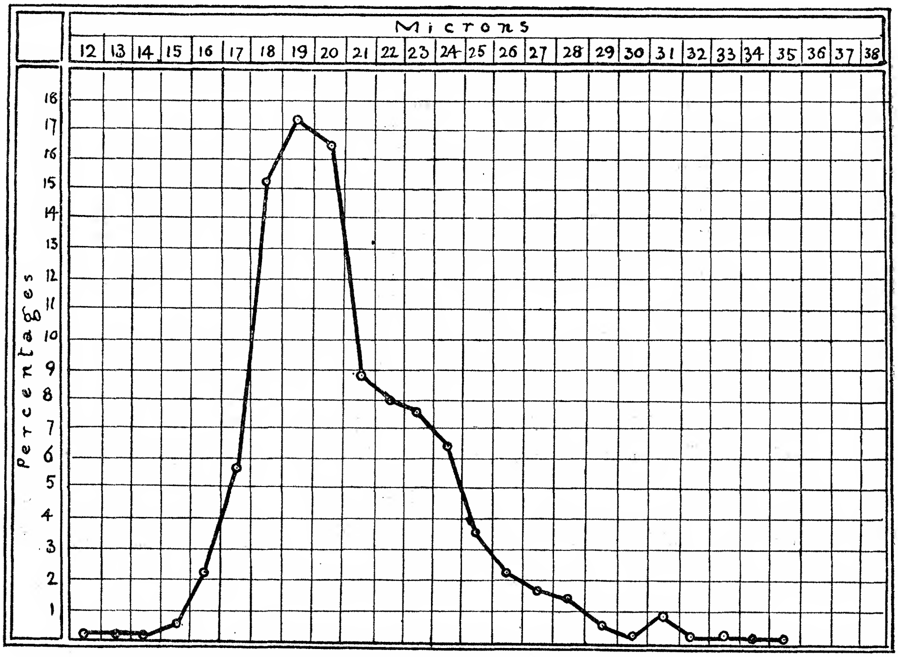
MORPHOLOGY OF THE LIWONDE STRAIN II.

Length.—The following table gives the length of this trypanosome as found in the white rat—500 trypanosomes in all :—

Table IV.—Measurements of the Length of the Trypanosome of the Liwonde Strain II.

Date.	No. of expt.	Animal.	Method of fixing.	Method of staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
1913.							
Aug. 18...	2363	Rat	Osmic acid	Giemsa	20·3	31·0	17·0
„ 18...	2363	„	„	„	19·0	23·0	14·0
„ 18...	2363	„	„	„	20·3	26·0	18·0
„ 19...	2363	„	„	„	19·2	25·0	18·0
„ 19...	2363	„	„	„	20·6	25·0	17·0
„ 19...	2363	„	„	„	19·7	24·0	17·0
„ 20...	2363	„	„	„	20·7	25·0	18·0
„ 20...	2363	„	„	„	21·2	29·0	18·0
„ 20...	2363	„	„	„	20·0	24·0	17·0
„ 21...	2363	„	„	„	19·0	24·0	16·0
„ 21...	2363	„	„	„	19·9	28·0	13·0
„ 21...	2363	„	„	„	19·8	26·0	17·0
„ 22...	2363	„	„	„	21·9	27·0	18·0
„ 22...	2363	„	„	„	21·9	28·0	18·0
„ 22...	2363	„	„	„	22·9	27·0	20·0
„ 23...	2363	„	„	„	22·6	33·0	18·0
„ 23...	2363	„	„	„	22·9	34·0	18·0
„ 23...	2363	„	„	„	21·0	30·0	17·0
„ 24...	2363	„	„	„	20·9	35·0	17·0
„ 24...	2363	„	„	„	22·2	31·0	16·0
„ 24...	2363	„	„	„	20·1	31·0	16·0
„ 25...	2363	„	„	„	20·9	28·0	16·0
„ 25...	2363	„	„	„	20·5	28·0	15·0
„ 25...	2363	„	„	„	20·6	27·0	12·0
„ 26...	2363	„	„	„	20·1	31·0	17·0
					20·7	35·0	12·0

CHART 2.—Curve representing the Distribution by Percentages, in respect to Length, of 500 Individuals of the Liwonde Strain II, taken on Nine consecutive Days, from Rat 2363.



Breadth.—The following table gives the breadth of this trypanosome in the rat—500 trypanosomes in all:—

Table V.—Measurements of the Breadth of the Trypanosome of the Liwonde Strain II, measured across the Widest Part, including the Undulating Membrane.

Experiment No.	Animal.	Number measured.	In microns.		
			Average breadth.	Maximum breadth.	Minimum breadth.
2363	Rat	500	3·0	4·75	1·25

Table VI.—Percentage of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of the Liwonde Strain II.

Date.	Experiment No.	Animal.	Percentage among short and stumpy forms.
1913.			
Aug. 18.........	2363	Rat	25
,, 19.........	2363	,, ...	13
,, 20.........	2363	,, ...	14
,, 21.........	2363	,, ...	16
,, 22.........	2363	,, ...	12
,, 23.........	2383	,, ...	23
,, 24.........	2363	,, ...	35
,, 25.........	2363	,, ...	37
,, 26.........	2363	,, ...	14
,, 27.........	2363	,, ...	23
		Average.........	21 ·2

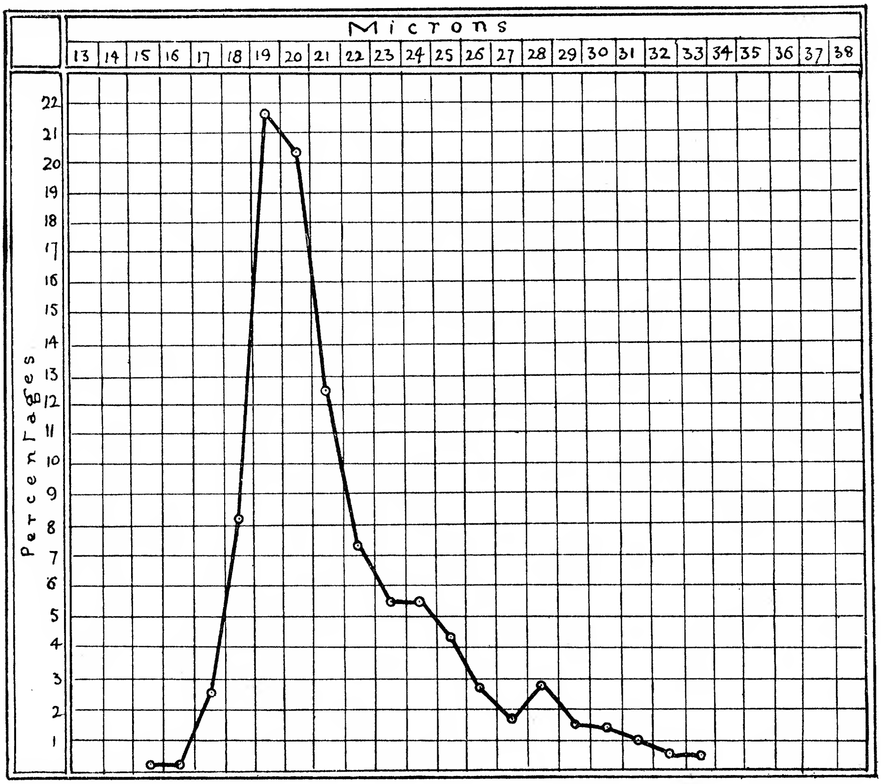
Morphology of the Liwonde Strain III.

Length.—The following table gives the length of this trypanosome as found in the white rat—500 trypanosomes in all:—

Table VII.—Measurements of the Length of the Trypanosome of the Liwonde Strain III.

Date.	No. of expt.	Animal.	Method of fixing.	Method of staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
1913.							
Aug. 18...	2370	Rat	Osmic acid	Giemsa	22 ·9	29 ·0	18 ·0
,, 18...	2370	,, ...	,,	,,	22 ·1	30 ·0	17 ·0
,, 18...	2370	,, ...	,,	,,	22 ·6	31 ·0	18 ·0
,, 19...	2370	,, ...	,,	,,	19 ·7	24 ·0	18 ·0
,, 19...	2370	,, ...	,,	,,	19 ·8	23 ·0	18 ·0
,, 19...	2370	,, ...	,,	,,	19 ·9	28 ·0	18 ·0
,, 20...	2370	,, ...	,,	,,	20 ·9	25 ·0	19 ·0
,, 20...	2370	,, ...	,,	,,	21 ·2	30 ·0	18 ·0
,, 20...	2370	,, ...	,,	,,	22 ·6	32 ·0	19 ·0
,, 21...	2370	,, ...	,,	,,	20 ·0	24 ·0	17 ·0
,, 21...	2370	,, ...	,,	,,	21 ·0	28 ·0	17 ·0
,, 21...	2370	,, ...	,,	,,	20 ·3	28 ·0	17 ·0
,, 22...	2370	,, ...	,,	,,	19 ·9	29 ·0	17 ·0
,, 22...	2370	,, ...	,,	,,	20 ·1	25 ·0	15 ·0
,, 22...	2370	,, ...	,,	,,	20 ·5	29 ·0	17 ·0
,, 23...	2370	,, ...	,,	,,	23 ·7	30 ·0	19 ·0
,, 23...	2370	,, ...	,,	,,	23 ·9	32 ·0	18 ·0
,, 23...	2370	,, ...	,,	,,	23 ·0	30 ·0	17 ·0
,, 24...	2370	,, ...	,,	,,	21 ·7	33 ·0	17 ·0
,, 24...	2370	,, ...	,,	,,	23 ·0	33 ·0	18 ·0
,, 24...	2370	,, ...	,,	,,	22 ·2	27 ·0	18 ·0
,, 25...	2370	,, ...	,,	,,	21 ·7	31 ·0	18 ·0
,, 25...	2370	,, ...	,,	,,	21 ·0	30·0	18 ·0
,, 25...	2370	,, ...	,,	,,	20 ·9	29 ·0	17 ·0
,, 26...	2370	,, ...	,,	,,	19 ·7	26 ·0	16 ·0
					21 ·4	33 ·0	15 ·0

Chart 3.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Liwonde Strain III, taken on Nine consecutive Days, from Rat 2370.



Breadth.—The following table gives the breadth of this trypanosome in the rat—500 trypanosomes in all :—

Table VIII.—Measurements of the Breadth of the Trypanosome of the Liwonde Strain III, measured across the Widest Part, including the Undulating Membrane.

Experiment No.	Animal.	Number measured.	In microns.		
			Average breadth.	Maximum breadth.	Minimum breadth.
2370	Rat	500	2 ·6	4 ·75	1 ·25

Table IX.—Percentage of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of the Liwonde Strain III.

Date.	Experiment No.	Animal.	Percentage among short and stumpy forms.
1913.			
Aug. 18.........	2370	Rat	4
„ 19.........	2370	„	44
„ 20.........	2370	„	36
„ 21.........	2370	„	33
„ 22.........	2379	„	22
„ 23.........	2370	„	4
„ 24.........	2373	„	6
„ 25.........	2370	„	12
„ 26.........	2370	„	30
„ 27.........	2370	„	33
		Average.........	22·4

Comparison of the Liwonde Strains with One Another.

Table X.—Measurements of the Length of the Trypanosome of the Liwonde Strains.

Date.	Experiment No.	Strain.	Animal.	Number of trypanosomes measured.	In microns.		
					Average length.	Maximum length.	Minimum length.
1913	2378	I	Rat	500	23·0	30·0	16·0
1913	2363	II	Rat	500	20·7	35·0	12·0
1913	2370	III	Rat	500	21·4	33·0	15·0
					21·7	35·0	12·0

Chart 4.—Curve representing the Distribution, by Percentages, in respect to Length, of 1500 Individuals of the Trypanosome of the Liwonde Strains.

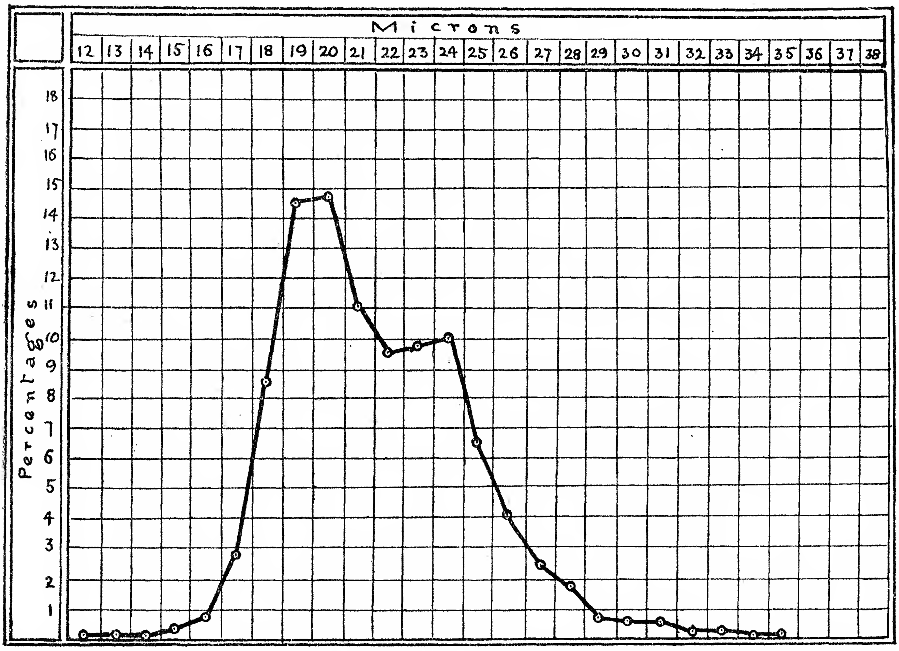


Table XI.—Measurements of the Breadth of the Trypanosome of the Liwonde Strains.

Date.	Experiment No.	Strain.	Animal.	Number of trypanosomes measured.	In microns.		
					Average breadth.	Maximum breadth.	Minimum breadth.
1913	2378	I	Rat	500	3·0	4·50	1·25
1913	2363	II	Rat	500	3·0	4·75	1·25
1913	2370	III	Rat	500	2·6	4·75	1·25
					2·9	4·75	1·25

Table XII.—Percentages of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Three Liwonde Strains.

Date.	Experiment No.	Strain.	Animal.	Percentage among short and stumpy forms.
1913	2378	I	Rat	15·8
1913	2363	II	,,	21·2
1913	2370	III	,,	22·4
			Average.........	19·8

COMPARISON OF THE LIWONDE STRAIN WITH THE HUMAN, WILD-GAME, AND WILD GLOSSINA MORSITANS STRAINS OF THE TRYPANOSOME CAUSING DISEASE IN MAN IN NYASALAND.

Table XIII.—Average Length of the Trypanosome of the Human, Wild-game, Wild *Glossina morsitans*, and Liwonde Strains.

Strain.	Number of trypanosomes measured.	Animal.	In microns.		
			Average length.	Maximum length.	Minimum length.
Human	5500	Rat	23·5	38·0	14·0
Wild-game	2500	,, ...	22·6	35·0	15·0
Wild *G. morsitans*	2500	,, ...	22·6	35·0	15·0
Liwonde	1500	,, ...	21·7	35·0	12·0
			22·6	38·0	12·0

Chart 5.—Curve representing the Distribution, by Percentages, in respect to Length, of 1500 Individuals of the Trypanosome of the Liwonde Strain, 5000 of the Wild-game and Wild *Glossina morsitans* combined, and 5500 of the Human Strain, all measured from Rats.

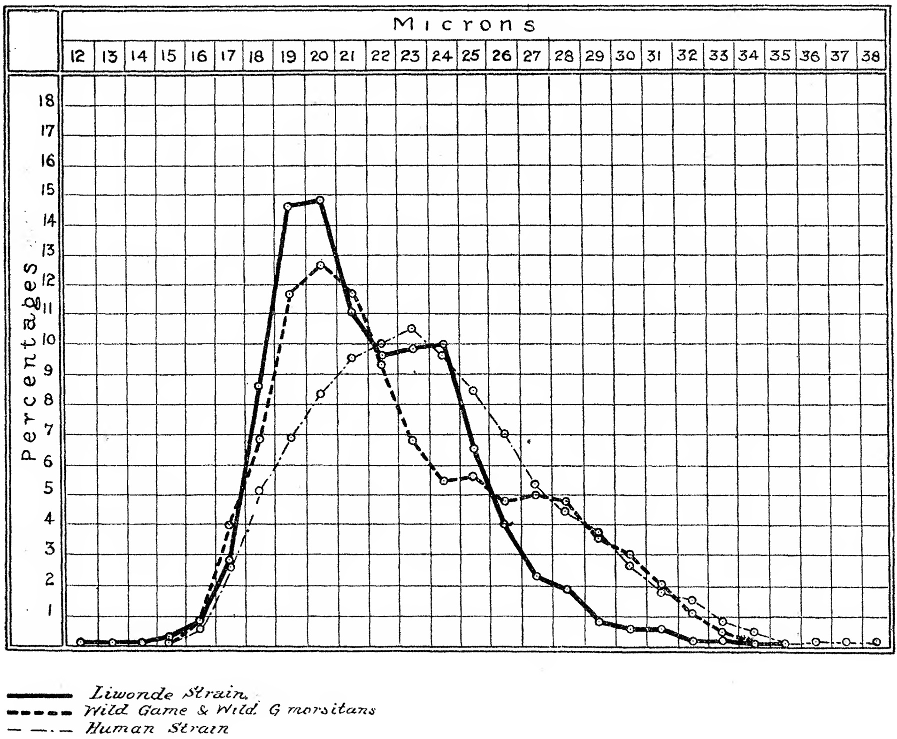


Table XIV.—Average Breadth of the Trypanosome of the Human, Wild-game, Wild *Glossina morsitans*, and Liwonde Strains.

Strain.	Number of trypanosomes measured.	Animal.	In microns.		
			Average breadth.	Maximum breadth.	Minimum breadth.
Human	1500	Rat	2 ·6	5 ·0	1 ·25
Wild-game............	1500	„	3 ·2	5 ·75	1 ·50
Wild *G. morsitans*	1500	„	2 ·9	5 ·25	1 ·25
Liwonde	1500	„	2 ·9	4 ·75	1 ·25
			2 ·9	5 ·75	1 ·25

Table XV.—Percentages of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of the Human, Wild-game, Wild *Glossina morsitans*, and Liwonde Strains.

Date.	Strain.	Animal.	Percentage among short and stumpy forms.
1912	Human	Rat.........	17·8
1912	Wild-game	„	26·2
1912	Wild *G. morsitans* ...	„	12·5
1913	Liwonde	„	19·8
		Average.........	19·1

II. *Animals susceptible to the Trypanosome of the Liwonde Strain.*

The following tables give the incubation and duration of the disease in goats, monkeys, dogs, guinea-pigs, and white rats, which were sub-inoculated with Strains I, II, and III from dogs infected in the Liwonde district:—

Liwonde Strain I.

Table I.

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
			Goat.		
1913. Aug. 12...	2382	Monkey 2340...	6	52	Died of Strain I.
„ 12...	2383	„ 2340...	6	44	„ „
			Monkey.		
Aug. 7...	2339	Dog 2322	4	27	Died of Strain I.
„ 7...	2339	„ 2322	4	72	„ „
			Dog.		
July 16...	2322	Wild flies	6	23	Died of Strain I.
Aug. 12...	2380	Monkey 2340...	6	46	„ „
„ 12...	2381	„ 2340...	6	45	„ „
			Guinea-pig.		
Aug. 12...	2384	Monkey 2340...	13	78	Died of Strain I.
„ 12...	2385	„ 2340...	23	114	„ „
			Rat.		
Aug. 12...	2378	Monkey 2340...	6	35	Died of Strain I.
„ 12...	2379	„ 2340...	6	44	„ „

* Duration includes the days of incubation; it dates from day of inoculation.

Liwonde Strain II.

Table II.

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
Goat.					
1913. Aug. 12...	2366	Monkey 2336 ...	6	39	Died of Strain II.
„ 12...	2367	„ 2336 ...	9	41	„ „
Monkey.					
Aug. 6...	2335	Dog 2323	5	32	Died of Strain II.
„ 6...	2336	„ 2323	5	43	„ „
Dog.					
July 5...	2353	Wild flies	3	—	Killed July 28.
„ 28...	2323	Dog 2353	9	17	Died of Strain II.
Aug. 12...	2364	Monkey 2336 ...	6	23	„ „
„ 12...	2365	„ 2336 ...	6	27	„ „
Guinea-pig.					
Aug. 12...	2368	Monkey 2336 ...	49	94	Died of Strain II.
„ 12...	2369	„ 2336 ...	—	—	Never showed trypanosomes.
Rat.					
Aug. 12...	2391	Monkey 2336 ...	5	22	Died of Strain II.
„ 12...	2363	„ 2336 ...	6	21	„ „

Liwonde Strain III.

Table III.

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
Goat.					
1913. Aug. 12...	2374	Monkey 2338 ...	6	10	Died of Strain III.
„ 12...	2375	„ 2338 ...	9	12	„ „
Monkey.					
Aug. 6...	2337	Dog 2321	5	32	Died of Strain III.
„ 6...	2338	„ 2321	5	38	„ „
Dog.					
July 8...	2356	Wild flies	4	—	Killed July 28.
„ 28...	2321	Dog 2356	9	16	Died of Strain III.
Aug. 12...	2372	Monkey 2338 ...	6	39	„ „
„ 12...	2373	„ 2338 ...	6	12	„ „
Guinea-pig.					
Aug. 12...	2376	Monkey 2338 ...	20	39	Died of Strain III.
„ 12...	2377	„ 2338 ...	73	93	„ „
Rat.					
Aug. 12...	2370	Monkey 2338 ...	6	20	Died of Strain III.
„ 12...	2371	„ 2338 ...	6	17	„ „

* Duration includes the days of incubation; it dates from day of inoculation.

Disease Set Up in Various Animals by the Trypanosome of the Liwonde Strain.

The infection set up in the various animals by the Liwonde strain gave rise to symptoms and appearances during life, and pathological changes in the various organs after death, alike and similar in every way to those caused by the Human strain, Wild-game strain, and the Wild *G. morsitans* strain of *T. brucei vel rhodesiense* found in the "Proclaimed Area."

COMPARISON OF THE THREE LIWONDE STRAINS IN REGARD TO THEIR VIRULENCE TOWARDS VARIOUS ANIMALS.

Table IV.—The Average Duration, in Days, of the Disease in various Animals.

Strain.	Goat.	Monkey.	Dog.	Guinea-pig.	White rat.
I	48	49	38	96	39
II	40	37	22	94	21
III	11	35	22	66	18

No recoveries took place among the experimental animals.

Table V.—The Average Duration of Life, in Days, of various Animals infected with the Liwonde Strain.

	Goat.	Monkey.	Dog.	Guinea-pig,	White rat.
Average duration, in days ...	33	41	28	84	26
Number of animals employed	6	6	9	5	6

COMPARISON OF THE LIWONDE STRAIN WITH THE HUMAN STRAIN.

Table VI.—The Average Duration of Life, in Days, of various Animals infected with the Human and the Liwonde Strains. The letter R stands for "refractory."

	Strain.	Ox.	Goat and sheep.	Baboon.	Monkey.	Dog.	Rabbit.	Guinea-pig.	White rat.
Average duration, in days.	Human	134	42	R.	26	34	28	67	30
Average duration, in days.	Liwonde	—	33	—	41	28	—	84	26

CONCLUSIONS.

1. The three wild *G. morsitans* strains from the Liwonde district resemble each other closely, and all belong to the same species of trypanosome.

2. The Liwonde strain belongs to the same species as that occurring in man, wild game, and wild *G. morsitans* inhabiting the "Proclaimed Area," Nyasaland—*T. brucei vel rhodesiense.*

3. Hence it would appear that wild *G. morsitans* occurring in a district 100 miles south of the "Proclaimed Area" are infected with the trypanosome which causes the human trypanosome disease of Nyasaland.

The Trypanosome causing Disease in Man in Nyasaland. The Naturally Infected Dog Strain. Part I.—*Morphology.*

By Surgeon-General Sir DAVID BRUCE, C.B., F.R.S., A.M.S.; Major A. E. HAMERTON, D.S.O., and Captain D. P. WATSON, R.A.M.C.; and Lady BRUCE, R.R.C. (Scientific Commission of the Royal Society, Nyasaland, 1912–14.)

(Received April 7,—Read June 25, 1914.)

[PLATES 9–11.]

INTRODUCTION.

This strain differs so much from the others that it is doubtful if it should be included among the various strains already described, Human,[*] Wild-game,[†] Wild *Glossina morsitans*,[‡] Mzimba,[§] etc. It has only been found on three occasions and, curiously enough, each time in a native dog.

The three dogs suffering from trypanosome disease were brought up to Kasu from the "Proclaimed Area," where they had probably been naturally infected by the wild *G. morsitans*, hence the name "The Naturally Infected Dog Strain."

All the infected dogs coming from this area did not show this strain; for example, Dog 553 was infected with a trypanosome resembling the ordinary Human strain.

[*] 'Roy. Soc. Proc.,' B, vol. 85, p. 423 (1912), and vol. 86, p. 285 (1913).

[†] *Ibid.*, B, vol. 86, p. 394 (1913).

[‡] *Ibid.*, B, vol. 86, p. 408 (1913).

[§] *Ibid.*, B, vol. 87, p. 26 (1913).